# Use of copper hydroxide in the cultivation of lisianthus seedlings (*Eustoma grandiforum* L.) under floating system

## Uso de hidróxido de cobre en plantines de lisianthus (*Eustoma grandiflorum* L.) cultivados en sistema de raíces flotantes

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### Abstract

Previous experiences demonstrated the efficiency of the floating system for growing lisianthus (*Eustoma grandiflorum* L.) seedlings. Using this system, root growth usually expands through the plug cell drainage hole into the solution originating some difficulties at transplant. Pruning roots could facilitate transplant as seedlings could be taken out of the tray more easily. However, root damages at the cutting point are a possible access for pathogens. The use of $Cu(OH)_2$ in the nutrient solution as a chemical pruning method was evaluated. Lisianthus seeds were sown in floating trays and placed in nutrient solutions containing 0, 12, 23, 46, 92 and 138 g $L^{-1}$ $Cu(OH)_2$. After 30, 40, 47 and 54 days, fresh and dry weights of seedlings were recorded. When seedlings reached four expanded leaves they were transplanted to soil in a greenhouse. At flowering, shoots were harvested to evaluate flower stem quality. Total root and shoot fresh and dry weights were higher for seedlings cultivated with 0.023 or higher doses of $Cu(OH)_2$. However, roots mass inside the tray cell were higher in all treatments compared to the control. The use of $Cu(OH)_2$ during plug stage improved transplant size and no effect on further plant growth and development was observed.

**Keywords**
floating bed • *Eustoma grandiflorum* L. • chemical pruning • propagation

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## Resumen

En el sistema de almácigos flotantes el crecimiento de las raíces generalmente excede el volumen de la celda pudiendo ocasionar algunas dificultades en el trasplante. La poda mecánica de raíces podría permitir que los plantines sean extraídos de la bandeja con mayor facilidad. Sin embargo, daños en las raíces debido al corte podrían ser vías de ingreso de patógenos. Semillas de lisianthus (*Eustoma grandiflorum* L.) fueron sembradas en bandejas flotantes utilizando soluciones nutritivas con 0, 12, 23, 46, 92 y 138 mg $L^{-1}$ de $Cu(OH)_2$ para evaluar su uso como agente de poda química. Luego de 30, 40, 47 y 54 días, se registró el peso fresco y seco de los plantines. Posteriormente se trasplantaron a un cantero dentro de un invernáculo y en floración se cosecharon para determinar la calidad de la vara. El peso fresco y seco de los plantines fue mayor al control en los tratamientos con 23 mg $L^{-1}$ o mayores concentraciones de $Cu(OH)_2$. Sin embargo, la masa de raíces dentro de la celda fue mayor en todos los tratamientos respecto del control. El uso de $Cu(OH)_2$ durante la producción de plantines mejoró el tamaño del plantín y no tuvo efecto en el crecimiento y desarrollo posterior del cultivo.



**Palabras clave**

almácigo flotante • *Eustoma grandiflorum* L. • poda química • propagación


## Introduction

The floating system is a widely used technique in the tobacco transplant production that replaces the conventional ground plant beds (14). Seeds are sowed in peat based media in polystyrene plug-trays floating in a nutrient solution tank during all the transplant production period. This technology has also been successfully used in the production of several horticultural transplants such as lettuce, paprika, tomato, cabbage, cauliflower, and more recently in ornamentals like gladiolus, liatris and lisianthus among others (4, 7, 8, 12, 14, 15, 19, 20, 23). In lisianthus (*Eustoma grandiflorum L.*) seedlings of greater size and higher root development were obtained with the floating system in a shorter period of time as compared to the conventional system (4).

In general, many authors mention a reduction in transplants production costs using the floating system as the application of mineral nutrients is easier and labor costs for watering can be eliminated. Also, bed disinfection is not needed and higher uniformity of transplants and greater survival rates can be obtained (3).

However, a negative point is that roots usually grow through the drainage hole of the tray into the nutrient solution. This causes difficulties when taking each transplant out of the tray and could also damage roots of the transplants at the moment of planting into the field (5). To avoid this problem, roots emerging from the drainage hole can be removed at the point of emergence. In a previous experiment we demonstrated that this removal does not affect the subsequent plant growth or development in lisianthus (5).

However, this practice could expose the damaged roots to the entrance of different pathogens.

The use of copper based components is a common practice to avoid the excessive growth of roots outside containers (17, 18, 22). This technique consists in painting the internal surface of containers with different $Cu(OH)_2$ or $CuCO_3$ doses (13). This pruning technique was evaluated in different woody and ornamental species (*e.g.* 1, 16) and in all cases transplants formed a better radical system with a better root distribution: more lateral roots were observed (1).

In this study we evaluated the application of different $Cu(OH)_2$ concentrations in the nutrient solution tank of a floating bed system as a method to avoid root growth through the drainage hole in the production of lisianthus transplants. This hypothesis is that the presence of $Cu(OH)_2$ in the nutrient solution would mimic the effects observed by painting the internal surface of containers, thus stopping root growth into the nutrient solution. The evaluation of possible negative effects of this compound on the subsequent plant growth under greenhouse cultivation was also evaluated.

## Materials and methods

Small nutrient solution tanks were simulated with 45 x 33 x 10 cm plastic containers. Expanded polystyrene trays of 288 cells were cut in three parts to obtain smaller trays of 22.6 x 11.7 x 6.1 cm with 96 cells each (17 $cm^3$). All containers were placed on a bench 1m above the ground inside a plastic greenhouse. Trays were filled with a commercial growing media (Grow Mix Tabaco A1, Terrafertil S. A., Argentina) containing 70% peat moss and 30% vermiculite and perlite. Lisianthus' Pink Picotee F1 seeds (Takii & Co. LTD., Kyoto, Japan) were sown on the trays and then placed into the containers with 12 L of reverse osmosis water (EC: 0.04 dS $cm^{-1}$; pH: 6). The water in the tank was replaced by a nutrient solution of 25 mg L-1 N (EC: 0.32 dS $cm^{-1}$; pH 6.23) from a 20 - 20 - 20 fertilizer (Peters Professional, Scotts Company, Ohio, USA) and then N content was increased to 50 mg $L^{-1}$ (EC: 0.59 dS cm-1; pH 5.98) after 30 days.

In order to check that root growth outside the drainage hole was occurring in all trays, the treatments were initiated when the first root tip was visible at the tray bottom. At that moment, six $Cu(OH)_2$ doses were applied at random in each tray: 0, 12, 23, 46, 92 and 138 mg $L^{-1}$ (7.5, 15, 30, 60, 90mg $L^{-1}$ Cu respectively). The addition of $Cu(OH)_2$ did not change EC or pH values of the nutrient solution in any of the treatments. The experiment was replicated 3 times in a complete randomized design. Each container with a polystyrene tray comprised an experimental unit.

The experiment was carried out in October 2011 and the average temperature was 25.1°C. Each tray received an average of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the light period (12 h).

Transplant production time was finished when 50% of transplants in each tray reached 4 expanded leaves (54 days after sowing). At that moment, fresh and dry weight of shoots and roots out of 10 randomly harvested transplants per treatment were registered. When root growth outside of the tray took place, root weight inside and outside the tray were recorded separately for each transplant. Atomic absorption spectroscopy (SpectrAA 220, Varian) was used to determine Cu content in seedlings' tissue.

Additionally, 21 transplants out of each tray were used to evaluate their subsequent growth at planting beds inside a plastic greenhouse. Transplants were planted at 63 plants·$m^{-2}$ in 0.9m width beds, grouped according to the $Cu(OH)_2$ treatment they received during transplant production and cultivated as single stems. Plants were daily irrigated and weekly fertilized with a commercial fertilizer 15-10-15 (Green Hakaphos, COMPO GmbH & Co. KG, Münster, Germany). At flowering, 15 plants per treatment were collected and stem length, fresh weight and number of nodes with flowers were registered. Data were analyzed with ANOVA and treatment means were compared with Tukey's test at $p < 0.05$ (9).

## Results

The first root tips outside the drainage hole were observed 30 days after sowing. At that moment $Cu(OH)_2$ was incorporated into the nutrient solution of the container according to the treatment concentration randomly assigned to each experimental unit.

At the end of the transplant production stage, root growth into the nutrient solution was reduced with the lowest $Cu(OH)_2$ dose tested (*i.e.* 12 mg $L^{-1}$) compared to the control treatment, and was totally avoided with higher concentrations (figure 1).

Using 23 and 46 mg $L^{-1}$ of $Cu(OH)_2$ increased total root dry weight of transplants compared to the control and the 12 mg $L^{-1}$ dose treatment, but this difference was not observed with higher $Cu(OH)_2$ doses (figure 2, page 65). Similar differences were observed among treatments when shoot dry weight or total dry weight were compared (figure 2, page 65).

At the end of the transplant stage, tissue Cu concentration in the control transplants was 7.2 mg $Kg^{-1}$ of total dry weight and no significant differences ($p = 0.1417$) were observed with other treatments (table 1, page 65).

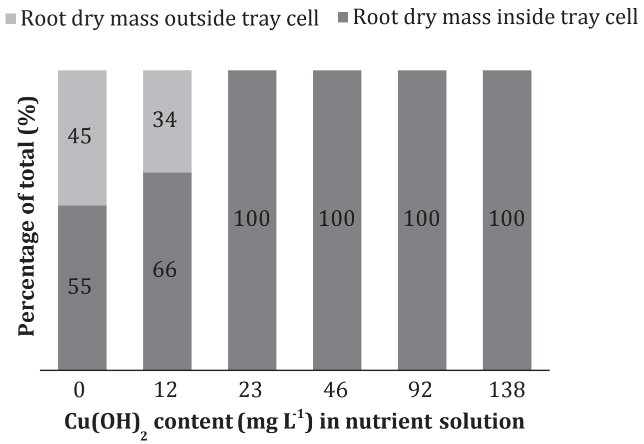
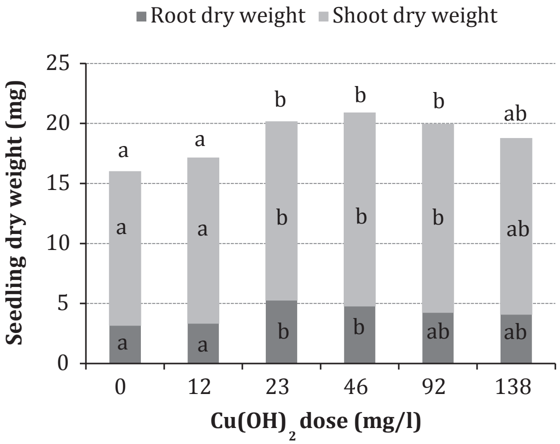



**Figure 1.** Fraction of roots out and inside the cell of transplants cultivated with different $Cu(OH)_2$ concentrations in the nutrient solution.

**Figura 1.** Fracción de raíces fuera y dentro de la celda de plantines cultivados con diferentes concentraciones de $Cu(OH)_2$ en la solución nutritiva.




Different letters between columns indicate significant differences by Tukey's test at P < 0.05.
Letras diferentes entre columnas indican diferencias significativas con test de Tukey (P < 0,05).

**Figure 2.** Root and shoot dry weight of transplants cultivated in a floating system with different $Cu(OH)_2$ concentrations in the nutrient solution.

**Figura 2.** Peso seco de raíces y tallo en plantines de lisianthus cultivados en sistema flotante con diferentes concentraciones de $Cu(OH)_2$ en la solución nutritiva.

**Table 1.** Tissue Cu content in lisianthus transplants cultivated with different concentrations of $Cu(OH)_2$ in the nutrient solution.

**Tabla 1.** Contenido de Cu en plantines de lisianthus cultivados con diferentes concentraciones de $Cu(OH)_2$ en la solución nutritiva.

| $Cu(OH)_2$ added to the nutrient solution (mg $L^{-1}$) | Cu in seedlings tissue (mg $Kg^{-1}$) |
|---|---|
| 0 | 7.2 a |
| 12 | 8.3 a |
| 23 | 8.2 a |
| 46 | 8.3 a |
| 92 | 8.2 a |
| 138 | 7.7 a |

The use of $Cu(OH)_2$ during the transplant production period did not affect subsequent plant growth and all plants from all treatments developed normally. At harvest, flower stem length, weight and number of nodes with flowers were similar among all treatments ($p > 0.05$; table 2, page 66).

**Table 2.** Length, weight and number of nodes with flowers of flower-stems harvested from plants cultivated with different $Cu(OH)_2$ doses during plug cultivation of seedlings under floating system technique.

**Tabla 2.** Largo, peso y número de nudos con flores de varas florales cosechadas de plantas cultivadas en soluciones nutritivas con diferentes concentraciones de $Cu(OH)_2$ durante la producción de plantines en almácigos flotantes.

| $Cu(OH)_2$ (mg $L^{-1}$) | Stem length (cm) | Stem weight (g) | Number of nodes with flowers |
|---|---|---|---|
| 0 | 29.4 a | 7.1 a | 5.8 a |
| 12 | 32.4 a | 8.4 a | 6.0 a |
| 23 | 29.9 a | 7.2 a | 5.8 a |
| 46 | 32.8 a | 8.1 a | 6.2 a |
| 92 | 28.2 a | 6.8 a | 5.1 a |
| 138 | 30.4 a | 7.4 a | 5.4 a |

## Discussion

Root growth outside the drainage hole was prevented in the production of tomato transplants cultivated in floating trays by paining the cell walls with a latex formulated with 7.1% $Cu(OH)_2$ (21).

In accordance to the results, this author observed that the dry weight of roots inside the cell was grater in trays treated than in those not treated with $Cu(OH)_2$. This was attributed to an increase in root branching after root tips stopped their growth when getting in contact with the Cu in the cell walls (6).

Among all $Cu(OH)_2$ doses tested, the minimal effective dose to prevent root growth outside the drainage hole was 23 mg $L^{-1}$. That minimal dose also permitted reaching the highest values of root and shoot dry weight of transplants so the use of higher doses seems to be unnecessary to control root growth of lisianthus, at least in the variety used in this experiment.

No toxicity symptoms were observed in transplant shoots of any of the treatments.

However, root tips of the control transplants were white, while those of other treatments were slightly brown. This could be due to a toxicity effect of Cu in the first millimeters of roots that were in direct contact with of Cu in the solution causing root development inhibition, thickening and darkening (1, 2, 24).

In several ornamental species the same root-tip darkening was reported when pruning roots with Cu compounds (2, 16, 18, 24).

Other visible Cu toxicity symptoms have been reported in leaves and shoots of other species when at least 20 mg $Kg^{-1}$ dry weight in plant tissue was registered (11), so according to the values measured in this experiment, Cu content all treatments of this experiment can be considered low in order to cause damages at aerial tissue level.

The presence of copper in the control treatment was due to the Cu content of the fertilizer used (0.02% as chelated Cu as indicated by fertilizer info tag).

In all other treatments, Cu concentrations measured in tissue at the end of the experiment were similar to the control indicating a possible regulation of Cu mobility from the solution to the plant as observed in recent reports. For example, Johnson and Singhal (2013) observed with an energy-dispersive X-ray analysis of root cell walls of *Lilium perenne* that the endodermis presented a barrier to the movement of free Cu ions.

According to our results, it could be recommended to incorporate 23 mg $L^{-1}$ Cu $(OH)_2$ in the production of lisianthus transplants as it was the lowest effective dose to stop the root growth into the nutrient solution.

However, the minimum effective dose should be evaluated before making any general recommendation as Cu concentration is species dependent. Arnold *et al.* (1993) reported best results when painting pot walls with added with concentrations between 25 to 50 g of $Cu(OH)_2$ $L^{-1}$, but Svenson and Johnston (1995) had to rise the concentration up to 100 g of $Cu(OH)_2$ $L^{-1}$ to obtain similar results in ornamental foliage species. Similar results were observed by Wyatt (1998) in tomato plant growth of transplants produced with or without Cu impregnation in cell walls. Ruter (1994) on the other hand, did find an increase in the vegetative development of chrysanthemum plants developed in pots containing Cu, respect to plants cultivated without Cu application.

It is concluded that the use of a low $Cu(OH)_2$ concentration (*i.e.* 23 g $L^{-1}$) in the nutrient solution is effective to avoid root growth outside the tray-cell and to increase total root and shoot transplant growth, facilitating transplant planting and avoiding damages in the roots that could lead to further pathogen infections.

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